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Melatonin – Therapeutic Role And Future In Reproductive Health

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Abstract: Melatonin is a lipophilic hormone, secreted by the pineal gland. Melatonin's major function is to control the circadian rhythm in humans, but throughout the years, experiments have provided proof of other functions, especially in the reproductive system. Melatonin's ability as a free radical scavenger and antioxidant has opened up many possibilities in reproductive health. Melatonin may be able to reduce infertility by delaying ovarian aging due to oxidative stress and also provide alternative treatments for PCOS, preeclampsia and endometriosis in females. Melatonin also has a potential in ART (assisted reproductive technology), where it can promote the production of good-quality oocytes and oocyte maturation. It might play a part in cryopreservation, due to its ability to protect frozen oocytes and embryos from ROS (reactive oxygen species). In males, melatonin can act as an antioxidant in ROS-sensitive germ cells, increase androgen synthesis and help in reducing infertility.

Keywords: melatonin, reproductive system, antioxidant, oxidative stress

1. Introduction:

Melatonin has been long known as the hormone that regulates the circadian rhythm in humans. Melatonin already has a therapeutic role in humans, melatonin pills are taken as a supplement to promote sleep and cure insomnia and jet lag. However, with several discoveries and researches on its role in the both male and female reproductive systems, melatonin, especially with its antioxidant activity has a much larger potential to be used in the field of reproductive medicine. This study explores the effect of melatonin on the reproductive system and the possible beneficial roles in which it can be used in future.

1.1.Role of melatonin as an antioxidant

Melatonin or N-acetyl-5-hydroxytryptamine is synthesized by cells of the pineal gland – the pinealocytes, mainly in response to darkness from the amino acid tryptophan.

Free radicals are highly reactive molecules with an unpaired electron that are produced as a byproduct of metabolism. Reactive oxygen species or ROS like superoxide (O_2-), hydroxyl (OH) is some of the most abundant free radicals in the body. These radicals collide with other molecules and donate or take an electron to achieve stability.

Accumulation of ROS can cause oxidative damage, leading to damaged DNA bases, mutations, cell cycle arrest, cells dysfunction Ing and ultimately apoptosis. Antioxidants are molecules that are capable of scavenging these ROS and the body needs to maintain the redox homeostasis between production and scavenging the ROS.

Cyanobacteria were one of the first organisms to synthesize oxygen. These organisms possessed and synthesized their own melatonin. The evolution of oxygen also led to the production of ROS, which led to the emergence of melatonin as an antioxidant molecule needed to eliminate these toxic ROS and free radicals. Therefore, melatonin's primary function was antioxidant activity, with rest of the physiological functions evolving over time (1).

Several experiments over the years have shown that melatonin is a highly potent free radical scavenger and antioxidant (Fig. 1). Melatonin acts as an antioxidant directly and quenches superoxide anion (O2•□), hydroxyl radical (•OH), hydrogen peroxide (H_2O_2), nitric oxide (NO), etc. When melatonin reacts with two –OH, cyclic-3-hydroxymelatonin (C_3OHM) is formed, which is the byproduct of antioxidant reactions andC_3OHM yields N1-acetyl-N2-formyl-5-methox ykynuramine (AFMK) and N1-acetyl-5-methoxykynuramine (AMK) and all three of these byproducts also act as antioxidants. In the cytosol, melatonin at concentrations higher than 1nM will react with calcium/calmodulin complex and inhibit NOS1 (nitrogen oxide synthase 1) mediated production of RNS (2).

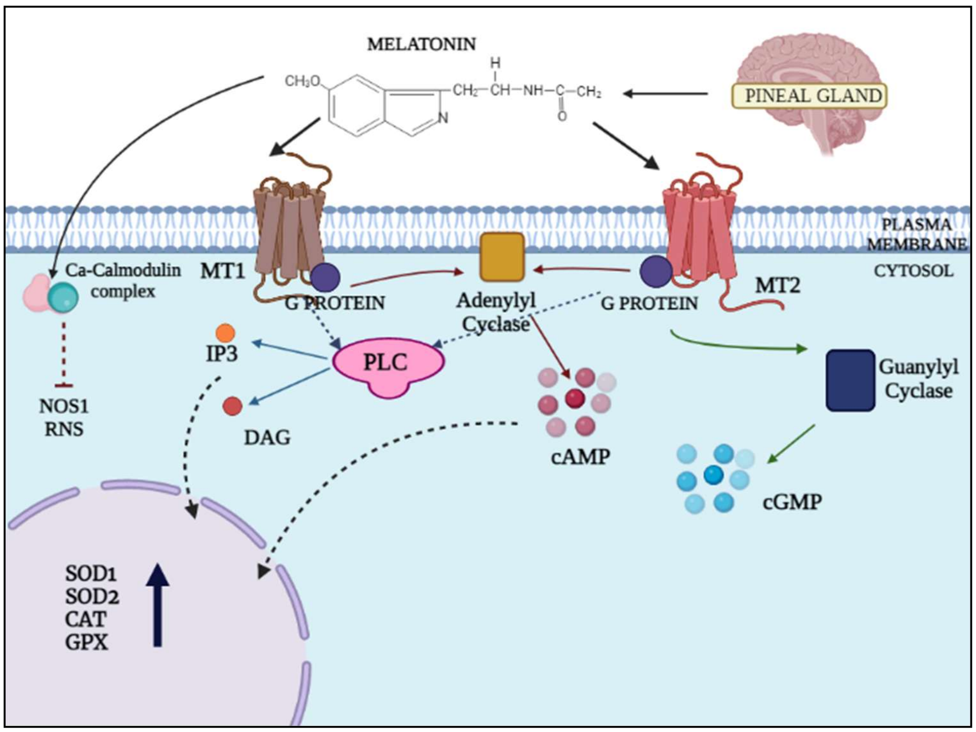

FIG.1. Melatonin's function as an antioxidant: Melatonin has two membrane bound receptors – MT1 and MT2 which are GCPRs and work by transducing signaling cascades and inducing the production of antioxidant enzymes like SOD, GPX and CAT. In the cytosol, melatonin reacts with calcium-calmodulin to prevent NOS1 mediated RNS production.
GPCR: G-protein coupled receptor; SOD: superoxide dismutase; GPX: glutathione peroxidase; CAT: catalase; ROS: reactive oxygen species; ROS: reactive nitrogen species; NOS1: nitrogen oxide synthase 1

Melatonin also acts indirectly on free radicals, where it stimulates antioxidative enzymes and increases their mRNA expression through MT1 and MT2 melatonin receptors, WHICH ARE G-protein coupled receptors and activate signaling cascades like cAMP (cyclic AMP) and IP_3-DAG (Inositol Trisphosphate-Diacylglycerol) mediated pathways , therefore increasing the total antioxidant count (TAC). These enzymes include superoxide dismutase (SOD), glutathione peroxidase (GPX), catalase (CAT) and glutathione reductase. It also increases the production of the potent free radical scavenger glutathione. Melatonin was found to neutralize reactive nitrogen species (RNS) like nitric oxide and the peroxynitrite anion and suppress the pro-oxidative enzyme nitric oxide synthase (3).

2. Melatonin in female reproductive health

2.1. Melatonin in the ovaries

Melatonin is present in high concentration in the follicular fluid and positively affects oocyte maturation. A large amount of ROS can be produced during ovulation, leading to DNA damage, mitochondrial dysfunction and lipid peroxidation in membranes and melatonin is hypothesized to act as an antioxidant to reduce oxidative stress in oocytes and granulosa cells. It is also known that the level of melatonin is decreased with age, with fertility decreasing too. Melatonin can promote oocyte maturation through MT1 receptor by decreasing the level of cAMP and promoting LH production. Melatonin also acts on follicular fluid to prevent apoptosis and follicular atresia. A study on follicular fluid collected during oocyte retrieval from women who have undergone IVF-ET, melatonin and 8-OhdG (8-hydroxy-2'-deoxyguanosine), a marker for DNA damage showed a negative correlation. Additionally, in patients receiving exogenous melatonin, the levels of melatonin in follicular fluid significantly increased and 8-OHdG concentrations were significantly decreased. Therefore, melatonin also has a protective function in the ovaries(4).

2.1.1 Melatonin in delaying ovarian aging

Fertility in women steeply declines after the age of35 years, rapidly decreasing from 40 years onwards, causing miscarriage, fetal aneuploidy and congenital defects in the newborns. Poor oocyte quality is a major reason for ovarian aging as women get older. This is caused due to oxidative damage caused by the presence of excessive levels of ROS and dysfunctional antioxidant defenses which fail to neutralize the ROS toxicity.

Although primarily secreted by the pinealocytes of pineal gland, in the female reproductive tract, melatonin is present in the follicular fluid produced by the ovarian and granulosa cells and is also taken up from the blood. Melatonin is known to be a potent antioxidant for eliminating ROS, so it plays an important role in the development of ovarian follicles and in regulating ovarian functions in different mammalian species including in humans. The level of melatonin decreases with increasing age. In a study on mice, it was discovered that maternal aging-induced loss of melatonin in follicular fluid resulted in the accumulation of ROS in oocytes (5). This led to failure in meiotic division and aneuploid eggs. Melatonin was supplemented both in vivo and in vitro. To determine the correlation between maternal aging and melatonin levels in-vivo, blood serum and follicular fluid was collected from both young and aged mice.

It was observed that melatonin levels in both blood serum and follicular fluid obtained from aged mice were quite lower than those obtained from young mice, showing that the levels of endogenous melatonin decrease with age. They also noted that the overall melatonin level was higher in the follicular fluid than in the blood serum, so a higher level of melatonin in the follicular fluid is required ovarian development and good oocyte quality. They tested for the ROS levels, to see if the decline in melatonin has had an influence on it. The data obtained showed that ROS signals were more upregulated in the aged oocytes than in the young oocytes. Apoptosis was also observed more frequently in aged oocytes.

Now, the researchers worked to see that if melatonin supplementation, given in vitro can alter the fate of aged oocytes and improve their quality by quenching the accumulated ROS. The oocytes were treated with melatonin in an in-trilateration medium for 12 hours, after which the ROS levels were tested in aged oocytes. Not only did they observe a decrease in ROS levels, but also observed a decrease in DNA damage, apoptosis, chromosomal defects and misaligned chromosomes. Next, to see if this could also be achieved by in-vivo administration of melatonin, they supplied aged mice with exogenous melatonin for 10 days and measured level of melatonin in blood serum and follicular fluid. Both showed an increase in melatonin levels. The same observations as the in-vitro treatment were observed with respect to ROS, with decrease in ROS levels, damaged DNA and apoptosis. So, we can see that supplementation of melatonin can be a potential tool not only to treat ovarian aging and infertility, but also might be successful in prolonging the period of fertility in women without the associated health risks to the mother and baby.

As mitochondrial function decreases with age, accumulation of ROS and oxidative stress causes a decrease in MMP (mitochondrial membrane potential) which can lead to the activation of cytochrome C, resulting in an apoptotic cascade. Studies have shown that melatonin can reduce the ROS level to maintain MMP and increase mitochondrial function in aged oocytes in in-vitro culture through the MT1/AMPK pathway (6).It was known through previous research that certain proteins like SIRT1, 2 and 3 acts as ROS sensors and played a role in quenching them and delaying postovulatory aging (7). The researchers identified Sirt1 gene of the Sirtuin family and to test whether melatonin uses this pathway to eliminate ROS, they tested its level of expression. They found that levels of Sirt1 were significantly decreased in aged oocytes but were recovered in the melatonin supplemented oocytes. Sirt1 regulates the activity of SOD2 (superoxide dismutase 2), which is a major antioxidant. SOD2 levels were decreased in aged oocytes and recovered in melatonin treated ones, just like the Sirt1 expression. This recovery was prevented if Sirt1 or melatonin were inhibited. Sirt1 inhibition also prevented the improvement in ROS levels, DNA damage, apoptosis and aneuploid oocytes on melatonin administration. Therefore, the observations proved that melatonin acts as an antioxidant by activating the Sirt1/Sod2 pathway.

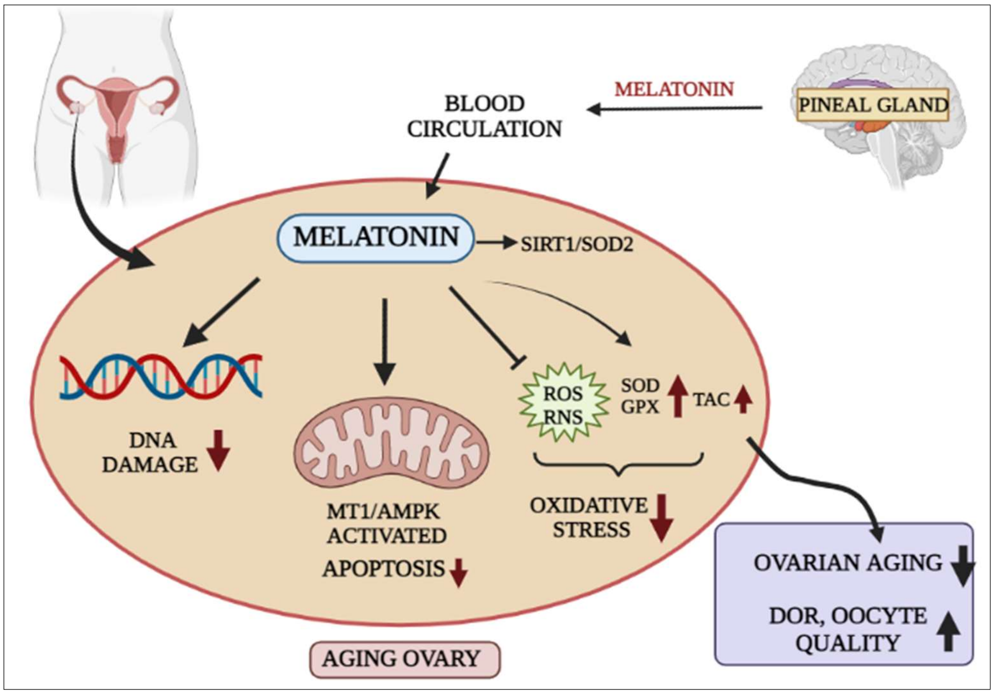

FIG. 2: Melatonin in delaying ovarian aging: Melatonin delays ovarian aging by antioxidant activities by activating Sirt1/SOD2 pathway, directly quenching ROS and RNS and indirectly increasing the levels of other antioxidants like GPX and SOD, also increasing the TAC, therefore preventing oxidative damage. It prevents DNA damage and apoptosis through the MT1/AMPK pathway, increasing mitochondrial function SOD- superoxide dismutase; GPX – glutathione peroxidase; TAC – total antioxidant capacity.

The follicles which survive in woman until puberty are the ovarian reserve. Diminished ovarian reserve (DOR) is a leading cause of infertility worldwide leading to loss of ovarian stimulation and pregnancy loss. Ovarian aging is the most common cause of DOR, other causes being genetics, stress or autoimmune disorders. DOR can also cause depression and cardiovascular disorders. DOR in early life can result in complete reproductive failure of women and there isn't a clear treatment to this problem yet. In a study to see if melatonin could help solve this problem, a group observed through the docking of hub genes that melatonin showed a high affinity to eight such hub genes (AKT1, EGFR, MAPK1, HRAS, SRC, ESR1, AR, and ALB). So, melatonin could have a beneficial role in improving DOR through these targets (8).So, these observations show that melatonin has the potential to reduce ovarian aging through various pathways. (Fig. 2)

2.2 Melatonin to treat reproductive diseases

2.2.1 PCOS

PCOS (polycystic ovarian syndrome) is a reproductive disorder in women which leads to the formation of cysts in the ovary. Women suffering from PCOS experience irregular and painful menstrual cycle, excessive hair growth and difficulty in pregnancy, often leading to premature infertility. Studies have shown that both the serum and follicular fluid of PCOS patients contains excess ROS and undergoes oxidative stress. This oxidative stress can cause may also lead to spindle disorders, DNA damage, and reduced mitochondrial function in oocytes ultimately resulting in poor quality oocytes and lower fertilization rates in PCOS patients. Melatonin's antioxidant function can help with these issues (Fig. 3), with melatonin directly quenching ROS and RNS and decreasing intracellular malonaldehyde (MDA) levels. (Fig. 3) Melatonin can also indirectly increase the production of other antioxidants like SOD and GPX. For example, glutathione (GSH) deficiency in PCOS patients leads to problems oocyte maturation, treatment with melatonin can be used to increase GSH levels (9).

Hyperandrogenemia is another symptom of PCOS which inhibits oocyte maturation. This overproduction of androgens is caused by the apoptosis of granulosa cells, which converts androgens to estradiol. Melatonin can help with hyperandrogenemia in two ways. Melatonin can upregulate B-cell lymphoma-2 (BCL-2), an antiapoptotic gene and downregulate B-cell lymphoma- 2-associated X (BAX) a pro-apoptotic gene, to prevent apoptosis of GC cells. Additionally, a study on mice showed mitochondrial swelling and membrane defect of GCs in PCOS patients. Treatment with melatonin upregulated SIRT1 protein expression which in turn activated PDK1/Akt (phosphoinositide dependent proteinkinase-1/Protein Kinase B) signaling pathway (Fig 3) , which is responsible for stabilizing the mitochondrial membrane and prevent membrane defects (10).

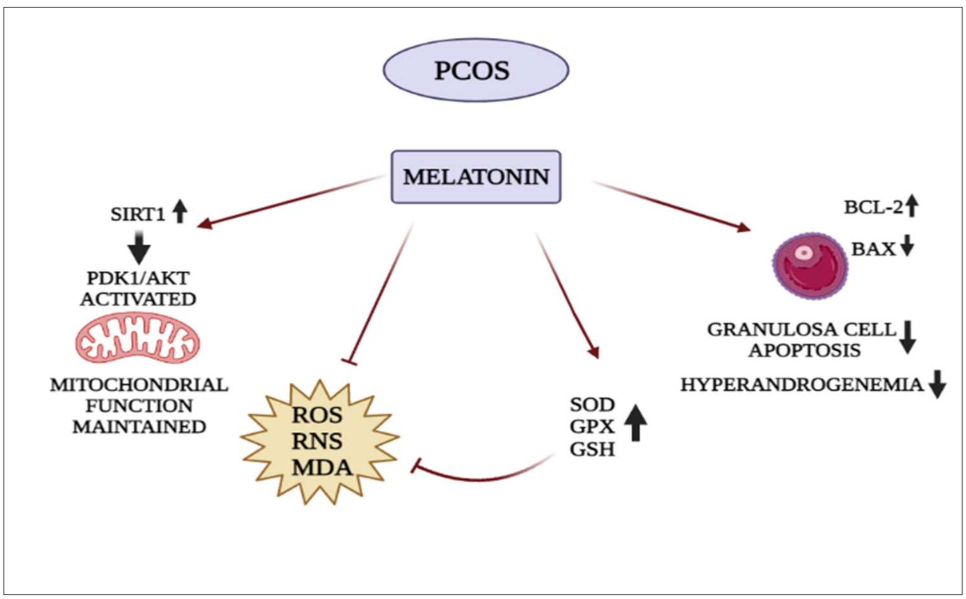

FIG. 3: Melatonin treatment in PCOS patients: Melatonin acts as an antioxidant by directly eliminating ROS and RNS, and increases antioxidant enzymes like GPX, SOD,and GSH. It can prevent hyperandrogenemia by upregulating BCL-2 and downregulating BAX, thus preventing granulosa cell apoptosis. Melatonin also maintains mitochondrial functioning through PDK1/AKT.
SOD: superoxide dismutase, CAT: catalase, GSH: glutathione: BCL2: B-cell lymphoma 2, BAX: B-cell lymphoma- 2-associated X

2.2.2. Preeclampsia

Preeclampsia is a disorder occurring in pregnant woman after 20 weeks of gestation characterized by abnormally high blood pressure in a previously normotensive patient, along with proteinuria and placental dysfunction.

In a study to investigate the link between melatonin and preeclampsia, the researchers first investigated the association between circulating levels of melatonin and preeclampsia, and secondly investigated the occurrence of preeclampsia in different seasons and if melatonin plays a role in this association (11).In women who had preeclampsia, the circulating levels of melatonin were found to be significantly reduced. Then, the circulating levels of melatonin in the 113 women who were affected by preeclampsia were measured. These circulating levels of melatonin were significantly lower in women who presented with preeclampsia in comparison to normal pregnant women. They also looked into expression of melatonin receptors (MT1 and MT2) in preeclamptic and normal placentae by immunohistochemistry and discovered a reduction in the expression of MT1 receptor in the preeclamptic placentae. These discoveries show that melatonin levels are reduced in preeclamptic women and melatonin treatment may present to be an option for the treatment of preeclamptic women someday.

2.2.3. Endometriosis

Endometriosis is a reproductive disease characterized by the growth of endometrial tissue outside the uterus like in the ovaries or fallopian tube. This causes pelvic pain, heavy menstruation, infertility and even an increased risk of cancer.

Researchers have seen that pinealectomy in rats caused higher lipid peroxidation and low antioxidant activity, which are indicators of endometriosis and this could be reversed by melatonin administration. It has also been observed through endometrial biopsies of affected women that the expression of melatonin receptors is varied. Other studies on mice showed that melatonin administration on endometriotic cells from severe combined immunodeficient mice decreased the COX2 (cyclooxygenase2) and MDA levels, and melatonin increased antioxidant levels (SOD and CAT - catalase) (12).

In spite of there being much prospect of melatonin as a treatment for endometriosis, there haven't been enough clinical trials yet.

2.3. Melatonin in assisted reproductive technology (art) and cryopreservation

ART or assisted reproductive techniques have changed the course of infertility; therefore, researchers are always looking for ways to increase the success rate of such techniques. IVF-ET (in vitro fertilization is a technique where the ovum is obtained from the woman and fertilized outside the body with the sperm, then the embryos are transplanted back into the uterine cavity.

Oxidative stress due to the presence of ROS in follicular fluid and blood serum can lead to poor quality oocytes, which might lead to reduced reproductive ability and infertility in women. Melatonin is an antioxidant and it has been known through previous studies melatonin treatment increased fertilization and pregnancy rate. In a study on patients with unexplained infertility (13), a decrease in the intrafollicular melatonin concentration was seen. Exogenous melatonin given at 3mg per day increased the levels of antioxidant activity to the level of those found in fertile women. The amount of good quality oocytes also improved, increasing the blastocyst count and number of transferable embryos in IVF-ET and Art success rates went up by 30%. They also did not find melatonin causing any adverse effects on these procedures.

A group of researchers conducted a study on whether melatonin treatment would increase clinical pregnancy rate and live birth rate in assisted reproductive technology (ART) cycles using 10randomized trials taken from 2010 to 2019 (14).In the procedure, they applied melatonin to different ART techniques, with its in vivo and in vitro administration for oocyte and embryo culture. They found ten studies showing an effect of melatonin on clinical pregnancy and meta-analysis showing that melatonin treatment caused a significant increase in the clinical pregnancy rate. On comparing the data of the number of good quality embryos which were available for extraction and synthesis in 3 studies, it was seen that melatonin treatment greatly increased the number of good quality embryo. Meta-analysis of the collected oocytes from 7 studies revealed that melatonin treatment significantly increased the number of oocytes collected. So, the findings of these studies show that melatonin does have an effect on ART procedures and might increase the success rate of such procedures significantly in the near future (Fig. 4).

Vitrification freezing is a technique of cryopreservation where cells of the oocyte or embryo are transformed into a vitrification state by adding a cryoprotectant, followed by ultrafast cooling. However, the method has its downsides, as the extreme temperature and toxicity of cryoprotectants can rupture the cell membranes and often damage the frozen oocytes. The blastocyst rate and the clinical pregnancy rate of cryopreserved oocytes are also lower than those of the fresh oocytes. A study showed that melatonin improves the effect of cryopreservation on human oocytes by decreasing oxidative stress and maintaining the permeability of the oolemma (15).

Melatonin application showed a decrease in early apoptosis, good quality embryo and increase in blastocyst rates. A study on bovine oocytes showed that when a cryoprotectant containing melatonin was used, apoptosis was prevented by decreasing BAX and BCL-2, decrease in ROS and Ca^{2+} levels. Furthermore, it was seen in mice that melatonin increased maternal to zygotic transition gene expression (MZT) (9). So, melatonin may be used in addition to cryoprotectants in vitrification freezing of human oocytes in the future (Fig. 3).

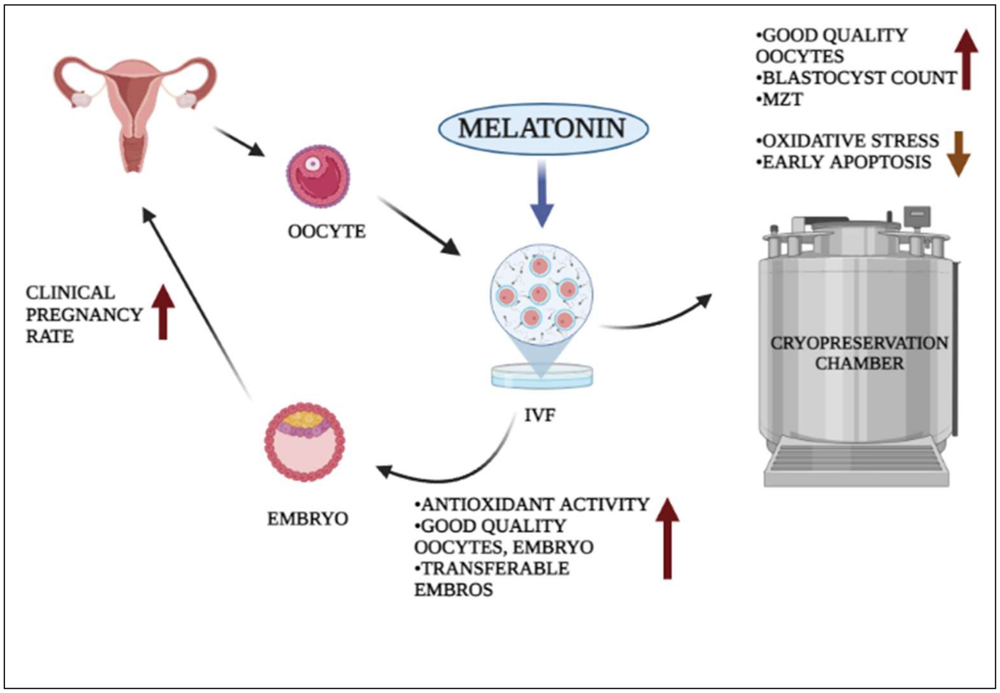

FIG.4: The role of melatonin in art and cryopreservation: In IVF-ET patients, melatonin supplementation can increase antioxidant activity and reduce oxidative stress, thus leading to the production of good quality oocytes and embryos and increase the number of transferable embryos. Therefore, it also increases the rate of pregnancy. Application of melatonin with cryoprotectants in cryopreservation can improve oocyte quality, blastocyst count and increase MZT.
ART: assisted reproductive techniques; IVF-ET: in vitro fertilization-embryo transfer;MZT: Mother to zygotic transition gene expression.

3. Melatonin in male reproductive health

Melatonin has both lipophilic and hydrophilic properties and is able to pass through the blood–testis barrier and enter the cells of the testis. There, it can modulate testosterone synthesis through coupling of G-protein with MT1 and MT2 receptors and regulate cAMP signal transduction pathways. Melatonin has been seen to act on two somatic cell types of the testis: Leydig and Sertoli cells. There was a decrease in Leydig cell apoptosis, as melatonin targeted the BCL2/BAX apoptotic pathway. Additionally, melatonin, through activation of cAMP, increased the expression of testosterone synthesis-related genes like Steroidogenic Acute Regulatory Protein (StAR), Steroidogenic factor 1 (SF1), and Transcription factor GATA-4 (Gata4) in Leydig cells, which was mediated by melatonin's nuclear receptor – RORα. All of these combined functions of melatonin in the Leydig cells lead to an increase in testosterone secretion, which is necessary for spermatogenesis in mammals(Fig. 5). In contrast, the knockout animals had dysfunctional Leydig cells and reduction of Star protein expression(16). In the Sertoli cells, melatonin exerts its antioxidant functions and maintains the balanced redox state. Melatonin has been found to increase the concentration of anti-oxidant enzymes SOD1, PXR1 (peroxiredoxin1) and CAT and decrease pro-apoptotic proteins in testicular mast cells in biopsies of infertile men. (17)

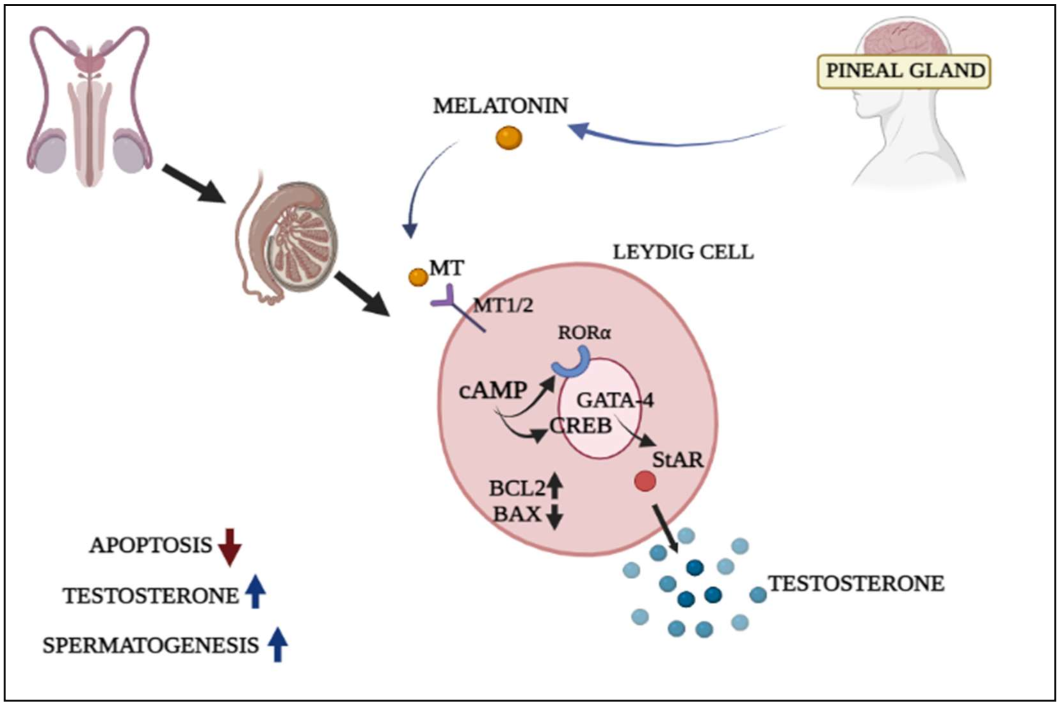

FIG. 5: The action of melatonin on Leydig cells: Melatonin increases testosterone synthesis and expression of testosterone synthesis-related genes like StAR and GATA-4 in Leydig cells through cAMP, mediated by nuclear receptor RORα.Melatonin also decreases Leydig cell apoptosis through regulation of BCL2/BAX.
StAR: Steroidogenic Acute Regulatory Protein; cAMP: cyclic AMP; BCL2: B-cell lymphoma 2, BAX: B-cell lymphoma- 2-associated X.

Other studies to see the protective effect of melatonin on human spermatozoa and to see if it could be applied to ART and cryopreservation were also performed. Oxidative stress due to production of ROS and lipid peroxidation during cryopreservation damages the sperm membrane and reduces the recovery of motile sperm. A study was performed to see if the antioxidative effects of melatonin caused a change in post-thaw motility, viability, and intracellular ROS and malondialdehyde (MDA) to human sperm freezing extender (18). The viability of sperm groups treated with the varying concentrations of melatonin significantly increased with respect to the control group. As MDA is the most abundant lipid peroxide, it was used to determine the levels of lipid peroxidation in sperms. The researchers measured the MDA concentration, and found an increase in all sperm groups after cryopreservation as compared to fresh sperms. They observed that the MDA level in melatonin treated groups was lower than that of the control group. So, these results show that melatonin added to human semen freezing extender can be beneficial in protecting sperms by increasing post thaw viability, motility and decreasing the ROS and lipid peroxidation.

4. Discussion:

In the above study, we have examined the various roles performed by melatonin and their effects in the reproductive system. Melatonin's ability as an antioxidant is perhaps the most important of all, and has a hand in reducing oxidative stress and protecting cell death and damage. This ability has led to it being a tool in decreasing and delaying ovarian aging, where it acts through the SIRT1/SOD2 pathway and eliminates ROS, RNS as well as increasing the TAC by activating other antioxidant enzymes and also increasing mitochondrial function through the MT1/AMPK pathway. This can not only help in improving the oocyte quality, but also increase diminished ovarian reserve, prolong the reproductive period in females and reduce infertility and subfertility. Melatonin can also be used as a treatment option for reproductive diseases like PCOS, preeclampsia and endometriosis which don't have a clear diagnosis till date. Melatonin's antioxidant functions could also be beneficial in ART, where it could help in producing good quality oocytes and number of transferable embryos and increase the success rate by increasing clinical pregnancies. Melatonin has been observed to have a protective effect on both oocytes and sperms in cryoprotection, where it protects them from oxidative stress, high temperatures that can rupture the cell membrane and toxic cryoprotectants and increase the success rate post-thawing. In males, melatonin also performs different functions on testicular somatic cells like Leydig cells where it influences testosterone production, spermatogenesis, cell proliferation and inflammation and in Sertoli cells where it increases antioxidant production. Even though there has been a lack of clinical trials in most cases, our observations show that melatonin could have quite an important therapeutic role in both male and female reproductive systems in the future.

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